

## 12 Sugar, syrups, and honey

### I Introduction

Sucrose, in commercial practice commonly referred to as sugar, is the most widely distributed sugar in nature and therefore easily manufactured in large quantities. Other simple sugars, such as dextrose (glucose), fructose, and lactose, or complex ones such as mannitol, sorbitol, or xylitol play also an important economic role.

Ninety nine percent of sucrose worldwide is derived from two main sources, sugar cane (*Saccharum officinarum*) and sugar beets (*Beta vulgaris*). Depending on the region, sucrose is also obtained from several types of palms (~1% of the worldwide production) such as the wild date palm (*Phoenix sylvestris*), coconut palm (*Cocos nucifera*), palmyra (*Borassus flabellifera*), and others; from sweet sorghum, *Sorghum bicolor* (L.) Moench (~0.05%); or from maple trees, *Acer saccharum* and *A. rubrum* (~0.01%) (FAO, 1998).

Fructose is obtained by ion-exchange separation from invert sugar, which is a hydrolysate of sucrose containing dextrose and fructose, or purified from high fructose maize (corn) syrup. Dextrose is manufactured from starch after complete hydrolysis, whereas lactose is commercially produced from whey. Polyols are usually manufactured by hydrogenation of easily available carbohydrates such as glucose syrup, fructose, or xylose derived from hemicellulose. Specifications for sucrose, dextrose, lactose, and fructose are given in the Codex Standards 4-1981, 7-1981 or 8-1981, 11-1981, and 102-1981 (Codex, 1994).

Sugar syrups are concentrated aqueous solutions manufactured from sucrose, from the sap of maple trees or obtained after hydrolysis of starches from potato, maize, or wheat. Specifications for glucose syrup are given in the Codex Standard 9-1981 (Codex, 1994). Honey is naturally produced by honey bees predominantly from nectars of flowering plants. Its sugar composition varies greatly according to the type of plant; specifications are provided in the Codex Standard 12-1981 (Codex, 1994).

### II Cane sugar

#### A Initial microflora

The numbers of microorganisms found on the surface of the green cane stalks and in exudates depend on the climatic conditions but *Bacillus* spp., *Enterobacter* spp., *Flavobacterium* spp., *Pseudomonas* spp., *Xanthomonas* spp., *Lactobacillus* spp., yeasts, and molds at levels ranging between  $10^3$  and  $10^9$  have been described (Nuñez and Colmer, 1968; Tilbury, 1970; Klaushofer *et al.*, 1998). Detailed studies showed that the composition of the populations were related to the sugar content and pH of the exudates (Duncan and Colmer, 1964; Bevan and Bond, 1971). The pink sugar cane mealybug (*Saccharococcus sacchari*), commonly found in sugar cane fields, excretes an acidic honeydew (pH ca. 3) which favors the development of acidophilic bacteria and yeasts (Ashbolt and Inkerman, 1990). After the mealybugs die, these microorganisms are replaced by less tolerant genera such as *Erwinia* spp. and *Leuconostoc* spp.. Damage to the cane caused by insects, frost, or other causes lead to the internal development of microorganisms. This development and in particular that of *Leuconostoc mesenteroides* which deposits dextran in the tissues are detrimental to sugar yields (Chen and Chung, 1993).

### B Effects of processing on microorganisms

The major stages and their implications for the microbiology of sugar cane manufacture are summarized in Table 12.1. Detailed reviews on sugar processing including recent developments have been published by Belotti *et al.* (2002) and Blackwell (2002).

**Harvesting.** Mature cane may be harvested green or after burning to remove the leaves, either manually with machetes (cane knives) or mechanically. Sometimes, cane that is cut mechanically is chopped into pieces (billets) of ~30 cm in length directly on the field, thus exposing many additional cut surfaces to contamination.

*Leuconostoc mesenteroides* is the microorganism of major concern since growth to high levels will lead to losses in the yield of sucrose of up to 1.5% due to formation of acids, dextran, and slime (Tilbury, 1975; Salunkhe and Desai, 1988; Cerutti de Guglielmone *et al.*, 2000). Tilbury (1970) found ~25% of the swab samples from cane stalks to contain *Leuc. mesenteroides* with levels ranging between <1 000 and 50 000. Levels can increase rapidly after burning, harvesting (Bevan and Bond, 1971), and growth. Thus, losses are minimized by rapid further processing. Trials with biocides to minimize losses have not been very successful (Desai and Salunkhe, 1991). In warm and dry conditions, yeasts may become the major microbial population (Lionnet and Pillay, 1988).

**Extraction and processing.** Cane is processed to raw sugar in a sequence of operations, which is outlined in detail by Desai and Salunkhe (1991) and van der Poel *et al.* (1998). These operations are: (i) cutting; (ii) crushing and milling to separate the raw juice from the bagasse; (iii) liming; (iv) heating; (v) clarification to separate the mud; (vi) evaporation to concentrate the juice; (vii) crystallization to obtain massecuite; and finally (viii) centrifugation. Most of these steps have an effect on the microflora.

**Table 12.1** Characteristics of the different steps in cane sugar manufacture

Process step	Temperature (°C)	pH	% Dry matter	Predominant microflora	Microorganisms	Results/problems
Post-harvesting	25–30	5.7–7.7	19	Mesophilic	<i>Leuconostoc</i>	Souring, Sugar loss/dextran formation
Crushing and extraction	25–30	5.0–5.6	10–18	Mesophilic	<i>Leuconostoc</i> , Enterobacter Yeasts	Souring, Sugar-loss, Alcohol production
Clarification	80–100	8.0	10–18	None	None	NA
Evaporation/ crystallization/ centrifugation	60–100	5.0–6.0	NA	None	Survival of thermophilic spores	NA
Microorganisms from Processing equipment						
Recontamination of raw sugar	25–30	5.6–6.0	70–90+	Osmophiles if $a_w > 0.65$	<i>Z. rouxii</i> , Xerophilic molds	Sugar loss, Invert sugars produced, Acid produced, $a_w$ rises
Refining	70–90	5.0–8.0	70–90+	None	Surviving thermophilic spores	NA
Refined sugar	25–30	N/A	99+	None	Surviving thermophilic spores	Introduction of spores into final products

Data from various sources.

N/A, not available.

During crushing and milling, the cane is shredded and passed sequentially through a series of rollers with extraction water flowing in the opposite direction. Juice from the first roller may contain up to 19% sugar, and from the last roller, <5%. The juice from the different rollers is combined to raw juice, and the extracted fibrous residue of cane is called bagasse. The raw juice is an ideal medium for growth of many microorganisms, but only a few compete successfully. Raw juice has a Brix (percent sucrose w/w, or equivalent in soluble solids) of 10-18, a pH of 5.0-5.6, is rich in inorganic and organic salts, amino acids, and other nutrients, and a temperature usually about 25-30°C. The bacterial count of the first expressed juice ranges between  $10^5$  and  $10^7$  per mL for normal cane and about  $10^8$  per mL for sour cane in which *Leuconostoc* spp. and other acid-producing microorganisms have multiplied, thus lowering the pH. The changes in the microbial flora during extraction and milling has been investigated by Lillehoj *et al.* (1984) with a special focus on *Leuconostoc* spp.

Recontamination with *Leuconostoc* spp. (which produce slime) is observed in mills where insufficient attention is given to hygiene. In particular, accumulation of bagasse produced during milling allows microbial development (Klaushofer *et al.*, 1998). In some mills, *Enterobacter* spp. predominate; in others, yeasts compete well.

The clarification process follows readily after extraction and permits minimalization of sugar losses unless it is delayed. It involves addition of lime to increase the pH to about 8.0 and rapid heating to 80-100°C to destroy vegetative microorganisms. Maintaining a high temperature in diffusion plants is an effective means of microbiological control. Sedimentation and filtration is then applied to remove scums, precipitates, and suspended solids as filter cake mud. Clarification decreases the microbial count by 99.999% (Table 12.2), but dextran and mesophilic or thermophilic spores remain (Chen and Chung, 1993). The clarified juice is then submitted to evaporation and crystallization at about 60°C to obtain a concentrated sugar suspension, the so-called massecuite. This is cleaned by centrifugation to remove the residual liquid phase, known as molasses, which consists of sucrose, inverted sugar, organic acids, amino acids, nitrogenous compounds, minerals, and polysaccharides, and to obtain raw cane sugar crystals.

Raw cane sugar is the end-product of the cane mill and the raw material for the refinery (Desai and Salunkhe, 1991; van der Poel *et al.*, 1998). It has a pH of 5.0-6.0, a water activity of about 0.65, and a sucrose concentration of 95-99%, with about 0.5% of residual molasses, which surround the crystals.

**Table 12.2** Bacterial content of sugar cane during processing<sup>a</sup>

Product	Mesophiles/mL (range)	Thermophiles/mL (range)
Raw juice (early) <sup>b</sup>	$8 \times 10^6$ – $1.6 \times 10^7$	$1 \times 10^1$ – $1 \times 10^2$
Raw juice (late) <sup>b</sup>	$6 \times 10^8$ – $8 \times 10^8$	—
Clarified effluent	0–11	0–8
Press juice	$0$ – $5 \times 10^4$	$3 \times 10^3$ – $2 \times 10^5$
Evaporator	$2 \times 10^2$ – $3 \times 10^4$	$2 \times 10^2$ – $2 \times 10^3$
Storage tank	$1 \times 10^3$ – $7 \times 10^3$	$2 \times 10^4$ – $4 \times 10^4$
Crystallizer	$2 \times 10^3$ – $4 \times 10^4$	$3 \times 10^2$ – $2 \times 10^4$
Masseccuite <sup>c</sup>	$1 \times 10^3$ – $1 \times 10^{4d}$	$2 \times 10^3$ – $2 \times 10^4$
Raw sugar	$3 \times 10^2$ – $5 \times 10^{3d}$	$2 \times 10^2$ – $2 \times 10^3$
Molasses	$3 \times 10^3$ – $3 \times 10^5$	$1 \times 10^3$ – $2 \times 10^4$

<sup>a</sup>Adapted from Owen (1977).

<sup>b</sup>Early in season and late in season.

<sup>c</sup>Mixture of sugar crystals and molasses.

<sup>d</sup>Per gram.

The microbial flora of raw sugar consists of bacterial spores, which will survive the thermal processes (Chen and Chung, 1993). Xerophilic yeasts are often present due to recontamination in storage tanks or by wet sugar residues which support their multiplication in the processing lines. This is often due to the poor hygienic design of equipment: long periods between cleaning and wet sugar residues favor growth to levels as high as  $10^6$  yeasts per gram (Tilbury, 1980). Several of these yeasts are also thermotolerant under these concentrated conditions (Bärwald and Hamad, 1984; Anderson *et al.*, 1988) and are often not killed during vacuum-pan crystallization. Air-borne molds such as *Aspergillus* and *Penicillium* spp. may contaminate products during crystallization, centrifugation, or drying.

Growth of yeasts in the molasses film is favored in inadequately centrifuged raw cane sugar or in case of moisture uptake. This may cause important economic losses due to the formation of invert sugar. Mold spoilage is, however, not of major concern, probably due to the absence of oxygen in silos as opposed to jute bags frequently used in the past.

**Refining.** The refining of raw cane sugar to food grade crystalline sugar is designed to remove impurities and produce crystals of sucrose >99.9% pure (Desai and Salunkhe, 1991; van der Poel *et al.*, 1998). The refining of raw cane sugar includes the following procedures which have an impact on the microbiological quality of the final product. During affination, raw sugar and molasses are separated by centrifugation and at the same time washed with water under high pressure. During this step, the residual molasses including microorganisms are eliminated. The washed sugar is then dissolved in hot water of about 70°C to obtain a 66° Brix syrup. This syrup is then mixed with lime and carbon dioxide, or phosphoric acid, to precipitate impurities, including bacteria, which are then removed by filtration.

Deionization is performed to remove ash followed by decolorizing through charcoal beds and ion exchange resins. The final steps, evaporation, crystallization, and drying, produce a crystalline sugar with levels of microorganisms ranging between <100 and <1000 cfu/g (Müller *et al.*, 1988).

### C Spoilage

Sour cane results when *Leuconostoc* spp. and other acid-forming bacteria grow in harvested cane (Tilbury, 1968; McCowage and Atkins, 1984). These bacteria produce invert sugar, lactic acid and acetic acid, and frequently dextran, which is used as an indicator of stale or old cane. Losses of sucrose may be substantial unless the time between harvest and crushing is minimized. In hot, humid climates, up to 15% of total sugar may be lost for each day between harvesting and crushing (Tilbury, 1975), whereas in warm but dry climates, the loss is usually much less and under such conditions ethanol is the preferred indicator (Lionnet and Pillay, 1988).

Dextran is a polysaccharide that causes significant processing problems for both raw sugar factories and refineries. Dextran increases the viscosity of the process liquid, necessitating slower processing. Dextran can damage pumps and can necessitate an increased frequency of cleaning of equipment such as vacuum pans. It also inhibits growth of sugar crystals and hence slows crystallization rates.

Dextran has an impact on yield and quality of sugar as well as on processing rates. Several publications have reviewed the causes and effects of this polysaccharide throughout the process (McCowage and Atkins, 1984; Clarke *et al.*, 1996; Clarke, 1997).

In cane diffusers, thermophiles ferment invert sugar to lactic acid (95%) and small quantities of formic acid, acetic acid, and glycolic acid. This is an important problem in cane processing (Oldfield *et al.*, 1974a; McMaster, 1975). The main acid producers are *Bacillus stearothermophilus* and *B. coagulans* (McMaster and Ravnö, 1977). In some cases, gas (carbon dioxide and hydrogen)

**Table 12.3** Minimum water activities permitting growth of osmophilic yeasts from raw sugars<sup>a,b</sup>

Yeast	Water activity
<i>Zygosaccharomyces rouxii</i>	0.65
<i>Saccharomyces bisporus</i> var. <i>mellis</i>	0.70
<i>Torulopsis candida</i> (1)	0.65
<i>Torulopsis candida</i> (2)	0.70
<i>Torulopsis etchellsii</i>	0.70
<i>Torulopsis versatilis</i>	0.70
<i>Hansenula anomala</i>	0.75

<sup>a</sup>From Tilbury (1967).<sup>b</sup>Tested in sucrose/glycerol syrups for 12 weeks at 27°C.

may be produced; and in battery diffusers, gas pressure may increase to levels affecting the sugar extraction.

Raw sugar is frequently stored for many months before shipping over long distances. Unless precautions are taken, spoilage may cause important economic losses.

Xerophilic yeasts appear to be most active but xerophilic molds may also cause, or contribute to, spoilage. The reasons for spoilage and the methods to prevent it follow.

Yeasts found in raw sugar are mainly xerophilic. Their natural reservoirs appear to be sugar cane, bagasse, filter cake mud, and wet sugary materials in the mill. *Zygosaccharomyces rouxii* is most common, but other species of *Zygosaccharomyces* and species of *Pichia*, *Candida*, *Dekkeroomyces*, and *Endomycopsis* have been found (Tilbury, 1968; Skole *et al.*, 1977). The minimum  $a_w$  permitting growth of some yeasts commonly found in raw sugar is given in Table 12.3.

Spoilage is caused by the growth of these xerophilic yeasts in the molasses film of the raw sugar and the rate of growth depends primarily on its water activity. The  $a_w$  of raw sugar varies widely, from 0.575 to 0.825. Spoilage does not occur  $<0.65$  but may progress rapidly at values  $>0.7$ . During storage, the increase of reducing (mainly invert) sugar and of the relative humidity of the atmosphere will cause an increase of  $a_w$  (Klaushofer *et al.*, 1998). During growth in the molasses film, the fructose component of the invert sugars is metabolized, and water and organic acids are produced. The increase of  $a_w$  and the decrease the pH favor further growth of xerophilic yeasts, and the decreased pH causes hydrolysis of sucrose to produce more invert sugar. Inversion may also result from the activity of invertase produced by a few xerophilic species of yeasts (Klaushofer *et al.*, 1998).

Under favorable conditions, growth of yeasts may continue during bulk storage and transport, and populations may reach  $10^7$ – $10^8$  cfu/g, levels affecting the organoleptic qualities of the final product. Within a few months after reaching the maximum, the population of viable yeasts may decline by  $>99.99\%$  (Tilbury, 1968).

The effect of different conditions and the interaction of different yeasts have been investigated under laboratory conditions and results published by Tilbury (1968).

The only refined product that has a history of spoilage is liquid sugar; granulated sugar rarely has been reported to be spoiled, and then only following accidental wetting (Müller, 1989). The main factor influencing its deterioration in storage is the temperature.

#### D Pathogens

Cane sugar has never been associated with food-borne poisoning outbreaks, and reference to *Salmonella* spp. in connection with microbiological criteria (Chen and Chung, 1993) is to be seen as part of the verification applied to raw materials used to manufacture other products. The processing and refining of sugar eliminate or destroy vegetative and probably also pathogenic microorganisms present in the

raw materials. Isolation of *Clostridium botulinum* from raw sugar and molasses, brown sugar lumps, and sugar used as bee-feed has been reported by Nakano *et al.* (1992). Spores of this pathogen were not detected in refined sugar or in samples taken during production, however.

## E CONTROL (cane sugar)

### Summary

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<b>Significant hazards<sup>a</sup></b>	<ul style="list-style-type: none"> <li>• No significant hazard.</li> </ul>
<b>Control measures</b>	
<i>Initial level (<math>H_0</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Reduction (<math>\Sigma R</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Increase (<math>\Sigma I</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<b>Testing</b>	<ul style="list-style-type: none"> <li>• In most situations, testing for <i>Salmonella</i> or hygiene indicators such as coliforms or Enterobacteriaceae is only performed as a verification for the adherence of good hygiene practices during processing and handling.</li> <li>• Testing for specific parameters is performed in special cases, for example, sporeformers in sugar used for canning.</li> </ul>
<b>Spoilage</b>	<ul style="list-style-type: none"> <li>• Growth of xerophilic yeasts possible if <math>a_w &gt; 0.65</math>.</li> </ul>

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<sup>a</sup>In particular circumstances, such as the use of sugar as ingredient for specific products or processes, other hazards may need to be considered.

### Comments

**Raw materials.** In the field, decreasing the interval between harvesting and processing decreases the opportunity for microbial growth. This interval should be not longer than 24–36 h for whole stalk cane and 8–12 h for chopped cane in hot humid weather or 18 h in cool dry weather. Cutting with sharp knives decreases ragged cuts and minimizes entry of bacteria through the cut surfaces. In mechanized harvesters, cleanliness and sanitizing of chopping boxes are desirable, but opportunities for such care are minimal.

**Measures applied during processing.** Application of formaldehyde to billets decreases spoilage but is not economical (Egan, 1971). Formaldehyde is also not an acceptable food process additive under US Food and Drug Administration regulations. During processing, it is essential to maintain good hygiene and to give sufficient attention to the cleanliness of equipment to avoid build-up of slime-forming bacteria (Klaushofer *et al.*, 1998). Accumulation of bagasse residues needs to be avoided and ideally mills should be steamed at regular intervals and cleaned during stops or shut-down. Growth and thus sugar losses are minimized by maintaining temperature in diffusers well above 70°C, preferably around 85°C. This is much more efficient than the use of expensive bacteriostats.

**Raw sugar.** The prevention of spoilage of raw sugar depends on obtaining and maintaining an  $a_w$  of <0.65. If this is achieved, the numbers and types of contaminants are of little consequence because they are unable to grow. Thus, procedures to control the water activity are important and are complemented by adherence to good hygiene practice. These steps are as follows.

- (i) Centrifugation sufficient to eliminate as much wash water as possible, to reduce any increase in  $a_w$ .

- (ii) Artificial drying of the raw sugar to an  $a_w < 0.65$ , if necessary.
- (iii) Storage in sealed silos at an RH below 65%.

*Storage stability of final products.* The overriding requirement is to avoid water uptake by the use of suitable packaging or storage under conditions of temperature and relative humidity that prevent the  $a_w$  rising  $>0.65$ .

### III Beet sugar

#### A Initial microflora

The microflora comes from the soil adhering to the beets. The genera identified from beet tissues are *Pseudomonas*, *Arthrobacter*, *Erwinia*, *Flavobacterium*, *Streptomyces*, and yeasts as well as mesophilic or thermophilic *Bacillus* spp. and *Clostridium* spp. (Bugbee *et al.*, 1976). *Bacillus* spp. are especially capable of causing spoilage during processing.

#### B Effects of storage and processing on microorganisms

The major processing steps and their impact to the microbiology of beet sugar are summarized in Table 12.4. Details on the processing steps are provided by Desai and Salunkhe (1991) and van der Poel *et al.* (1998).

*Storage, fluming and washing.* Beets are harvested before the onset of winter. After topping and cleaning, they are often stored in piles on the field for several days to months. Normally, a properly covered and ventilated pile will maintain a temperature of about 1.5–5°C even if the external temperature drops as low as –35°C.

If piles are not well prepared, sugar beets may spoil due to damages caused by freezing or overheating to temperatures ~50°C. Beets stored without adequate air circulation, mainly due to pockets of trash or earth, may overheat to ~50°C within 2 days and thereafter show evidence of microbial spoilage

**Table 12.4** Characteristics of the different steps in beet sugar manufacture

Process step	Temperature (°C)	pH	%Dry matter	Predominant/microflora	Microorganisms	Results/problems
Beet storage	0–15	7.5	25	Psychrophilic/mesophilic	<i>Bacillus</i> spp., <i>Leuconostoc</i> spp.	Slime formation, dextrans, levans
Beet fluming	0–15	7.5–9.0	N/A	Psychrophilic	<i>Pseudomonas</i> spp., <i>Flavobacterium</i> spp.	Acid production, corrosion
Beet washing	0–15	7.0	N/A	Psychrophilic	As above	As above
Extraction system	70	6.0–6.5	0.5–15	Thermophilic	<i>B.stearothermophilus</i> , <i>Clostridium</i> spp., thermophilic cocci	Acid production, sugar loss, hydrogen sulfide production
Raw juice system	35–40	6.0–6.5	14–15	Mesophilic		None
Raw juice cistern	25 or 55	6.0–6.5	14–15	Meso/thermophilic		None
Preliminary	60	6.0–11.0	14–15	Thermophilic	<i>B.licheniformis</i>	Acid production, sugar loss
Mainlining	80	12.5	14–15	None		
Thin juice system	70–128	9.2	14–15	None		
Thick juice system	70	8.6–8.8	70	None		
Boiling crystallization	70–80	8.8	70–92	None		
Crystal/syrup	40–50	7.2	99/90	None	Survival of thermophilic spores	Introduction of spores into final products

Adapted from Nystrand (1984).

N/A, not available

(Bugbee and Cole, 1976; Cole and Bugbee, 1976). Spoilage leads to the production of dextran, levan, or inverted sugars (Oldfield et al., 1971; Cole and Bugbee, 1976).

At the factory, beets are flumed in water to washers at 30-40°C, where residual soil is removed. Microbial numbers can be reduced by using fresh or chlorinated recirculated water (Carruthers and Oldfield, 1955; Moroz, 1963). Residual soil and leaching of sugar allow rapid growth to levels of  $10^6$ – $10^7$  bacteria/mL. In general, the higher the bacterial population in the flume water, the higher the population in the juice from the beets.

**Extraction.** Beets are cut into cossettes (thin V-shaped strips) and extracted with hot water in counter-current diffusers.

Diffusers normally have two ancillary systems, one for the extraction water and one for the juice, that pump, transport, filter, strain, heat, and store liquids generated during the process. Details of various beet processing lines are provided by Salunkhe and Desai (1988).

In the diffusers, gradients of sucrose (from 0.5% at the tail to 15.0% at the head) invert sugars, minerals and nitrogenous compounds, the temperature (25-75°C), dissolved oxygen, or the pH (5.0-8.0) occur.

The microorganisms that enter the head end of the diffuser reflect the microflora of the soil in which the beets were grown. However, in these gradients only a few groups of microorganisms will proliferate. As shown by Hollaus *et al.* (1997), the majority of the flora is composed of mesophilic microorganisms, mainly *Lactobacillus* and *Leuconostoc* spp., although coliforms may be present also. Yeasts may grow well in some places but, in general, molds and yeasts do not grow sufficiently to cause losses of economic consequence.

The few thermophilic microorganisms are of particular significance. In the part of the extraction plant where temperatures reach 65-75°C, growth of *Bacillus stearothermophilus* or *B. coagulans* is favored (Klaushofer *et al.*, 1971). Counts may reach levels of  $10^6$ – $10^7$  cfu/mL within few hours and produce sufficient acid to reduce the pH from 6.5 or 7.0 to 5.2-5.4 (Oldfield *et al.*, 1974a). However, in more anaerobic extraction systems such as batch diffusers, thermophilic *Clostridium* spp., some producing hydrogen sulfide, may grow (Belamri *et al.*, 1991, 1993; Pollach *et al.*, 2002). If lower temperatures are used, the losses of sucrose during extraction can be high.

Avoiding decrease in pH is important. For example, a decrease of 0.5 pH units below 6.5 may mean that *Bacillus* spp. become dominant, and a sugar loss of 0.16-0.19% may occur. The corresponding figure for *Clostridium* spp. is 0.10-0.13% (Hollaus, 1977).

The water used in the diffusers is either fresh or recycled from the last extraction step. This recycled water still contains 0.5-1.0% recoverable sucrose allowing for growth of microorganisms present in pipes, strainers, filters, tanks, or diffusion cells. Development can be avoided by heating the water to >80°C or by using biocides (Brigidi *et al.*, 1985; Franchi and Bocchi, 1994).

**Liming, carbonation, evaporation, filtration, and crystallization.** The raw juice is heated to 80-90°C and lime added as an aqueous suspension (milk) or as a slurry of calcium saccharates to precipitate colloidal material. Carbon dioxide is introduced (as bubbles) in two steps, first to improve removal of sludge and second to precipitate residual lime. Precipitates are eliminated during filtration and the filtrate (thin juice) is treated with sulfur dioxide to lower the pH and destroy colors. The juice is then submitted to ion exchange before being evaporated to obtain thick juice with a water activity of 0.88. The juice is then filtered and finally the standard liquor obtained is crystallized to raw beet sugar or massecuite. The microbiology of the concentrated extract has been reviewed by Hein *et al.* (2002). Details of different industrial processes are provided by Desai and Salunkhe (1991) and van der Poel *et al.* (1998).

**Refining.** There are three main steps during which microbial growth may occur: in deionization beds, charcoal beds, and sweet waters. Deionization of clarified liquor is carried out at low density (55°Brix) and at a temperature (50°C) that permits growth of thermophiles. In charcoal beds,  $a_w$  and temperature

preclude growth, but elution of sugar before regenerating the charcoal beds by heating at high temperatures may allow growth of thermophilic *C. thermosaccharolyticum* that are able to produce large quantities of gums and slimes (Belamri *et al.*, 1991).

Sweet waters are sugar-containing waters from several sources including bag washers, dust collectors, spillage, and wash water from filters, charcoal decolorizing beds, and deionizers. The pH ranges from 4.5 to 7.5 (usually about 5.5), the Brix from 0° to 60°, and the temperature from 15°C to 75°C. The temperature determines the type of microorganisms able to grow and mesophilic or thermophilic bacteria including *Leuconostoc*, *Lactobacillus*, *Streptococcus*, and *Bacillus* spp. at levels of  $10^4$ - $10^7$  cfu/mL have been described (Tilbury, 1975; Tilbury *et al.*, 1976). The yeasts most frequently present are *Candida*, *Zygosaccharomyces*, and *Pichia* spp., but others may be present also. Many are xerophilic and some actively produce invertase. At suitable temperatures, there may be  $10^5$ - $10^6$  yeasts/mL. Because sweet waters are used during refining, for example, to melt sugar, or to dilute high Brix solutions, the microbial quality of sweet waters is important.

Spore-forming bacteria compete well in conditions that are unsuitable for asporogenous lactic acid bacteria. They survive high temperatures and are usually the only microorganisms present when  $a_w$  and temperature become suitable for microbial growth. This is reflected in the flora of the refined sugar which is mainly formed by mesophilic or thermophilic, aerobic or anaerobic *Bacillus* spp. or *Clostridium* spp. (Hollaus, 1977; de Lucca *et al.*, 1992; Hollaus *et al.*, 1997). Other sources of recontamination include the air, contaminated dust, or packaging material (Pollach *et al.*, 1998).

### C Spoilage

During the extraction process, microbial activities create the following problems.

*Formation of acids and sugar losses.* The pH of raw juice ranges between 6.0 and 6.7. Microbial growth and acid formation during extraction are the main causes of sugar losses. Species vary widely in their ability to degrade sucrose (Table 12.5). Thermophilic bacteria, for example, growing at 70°C in diffusion juice may reach viable populations of  $10^6$ - $10^7$  cfu/mL and reduce the pH to 5.2-5.4. Numerous studies on the metabolism of microorganisms growing in diffusers and their impact on sugar losses have been recorded. Results are summarized and discussed by Klaushofer *et al.* (1998).

**Table 12.5** Rate of destruction of sucrose by microorganisms from beet juice

Organism	Temperature (°C)	Sucrose destroyed (mg/10 <sup>9</sup> cells/h)
<i>Desulfotomaculum (Clostridium) nigrificans</i> <sup>a</sup>	55	0
<i>Enterobacter (Aerobacter) aerogenes</i>	35	0.1-0.4
<i>Flavobacterium, Micrococcus</i> , <sup>b</sup>		
<i>Streptococcus</i> <sup>b</sup>		
<i>Leuconostoc mesenteroides</i> <sup>b</sup>	35	2-8
<i>Lactobacillus</i> <sup>b</sup>		
<i>Clostridium thermohydrosulfuricum</i> <sup>a,c</sup>	66	2-3
<i>Clostridium thermohydrosulfuricum</i> <sup>a,c</sup>	70	7-8
<i>Clostridium thermosaccharolyticum</i> <sup>a</sup>	66	2-3
<i>Bacillus thermophilus</i> <sup>b,c</sup>	55	10-40
<i>Bacillus stearothermophilus</i> <sup>a</sup>	65	108-160
<i>Bacillus subtilis</i> <sup>b</sup>	55	20-60
<i>Saccharomyces</i> <sup>b</sup>	35	1500-3000

<sup>a</sup>From Klaushofer and Parkkinen (1966).

<sup>b</sup>Devillers (1955).

<sup>c</sup>Not listed in the eighth edition of Bergey's Manual (Buchanan and Gibbons, 1974).

**Corrosion.** Steel in diffusers and ancillary systems corrodes from reaction with lactic acid. The rate of corrosion at 70°C is about twice that at 20°C and increases ~4-fold for each decrease of 1 pH unit in the range 6.2–4.2 (Allen *et al.*, 1948; Carruthers and Oldfield, 1955). Using lime to increase the pH of the diffusion water in the supply tank, decreases the overall corrosion rate but increases the depth of pitting. Inhibition of microbial growth by chlorination of the diffusion water inhibits corrosion in the recirculation system but not in the diffusers.

**Formation of slime.** Growth of mesophilic microorganisms such as *Leuconostoc mesenteroides* is only possible in cases where the temperature falls <40°C, e.g. in the countercurrent juice/cosette heat exchanger, at the exhausted pulp outlet or in the pulp presses. This is, however, well known now and only occurs exceptionally (Chen and Chung, 1993; Tallgren *et al.*, 1999).

**Formation of nitrite.** Nitrate is present in sugar beets, usually at 20–200 ppm of nitrate nitrogen; nitrite is absent. In raw juice, the nitrite level is usually 2–15 ppm, occasionally up to 75 ppm. In continuous diffusers, *B. stearothermophilus* is the prevalent thermophile, which reduces nitrate to nitrite or to nitrogen gas depending on the strain. Reduction of nitrate to nitrite at different steps of the process by *Thermus*, a highly thermophilic non-sporing Gram-negative bacterial genus, has been described by Hollaus *et al.* (1997).

Nitrite formed by these microorganisms may combine with other chemicals such as bisulfite formed from sulfur dioxide added during processing (Carruthers *et al.*, 1958; Oldfield *et al.*, 1974b). Nitrite combines with the bisulfite, reducing its efficacy and also forms imidodisulfonate which co-crystallizes with sucrose, thus increasing the ash content and causing malformed crystals that impede centrifugation of the massecuite.

## D Pathogens

As for section II.

## E CONTROL (beet sugar)

### Summary

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<b>Significant hazards<sup>a</sup></b>	<ul style="list-style-type: none"> <li>No significant hazard.</li> </ul>
<b>Control measures</b>	
<i>Initial level (<math>H_0</math>)</i>	<ul style="list-style-type: none"> <li>Does not apply.</li> </ul>
<i>Reduction (<math>\Sigma R</math>)</i>	<ul style="list-style-type: none"> <li>Does not apply.</li> </ul>
<i>Increase (<math>\Sigma I</math>)</i>	<ul style="list-style-type: none"> <li>Does not apply.</li> </ul>
<b>Testing</b>	<ul style="list-style-type: none"> <li>In most situations, testing for <i>Salmonella</i> or hygiene indicators such as coliforms or Enterobacteriaceae is only performed as a verification for the adherence of good hygiene practices during processing and handling.</li> <li>Testing for specific parameters is performed in special cases, for example, spore-formers in sugar used for canning.</li> </ul>
<b>Spoilage</b>	<ul style="list-style-type: none"> <li>Growth of xerophilic yeasts possible if <math>a_w &gt; 0.65</math>.</li> </ul>

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<sup>a</sup>In particular circumstances, such as the use of sugar as ingredient for specific products or processes, other hazards may need to be considered.

### Comments

*Raw materials.* The microbial content of flume water may be reduced by using fresh instead of recirculated water; however, this is not usually practical. Heavy chlorination may be useful in flumes carrying damaged beets (Moroz, 1963), but chlorination does not destroy spore-forming thermophiles (Carruthers and Oldfield, 1955).

*Measures applied during processing.* Ideally, the temperature in diffusers and ancillary systems should be at 75°C throughout to minimize microbial growth. At 70°C, abundant growth of *B. stearothermophilus* may occur; whereas at 80°C, excessive extraction of pectin may interfere with clarification. If extraction is performed at temperatures >70°C, stagnant regions in the pipelines and extraction plant are eliminated and minimal sugar losses are accepted, then operation without formalin is possible (Hollaus and Pollach, 1993). However, when extraction is performed at temperatures between 60°C and 72°C, then addition of bactericides is an essential part of the control measures. Several preservatives are effective, e.g. quaternary ammonium compounds, benzoate, formaldehyde, and metabisulfite. Of the legally permissible compounds, metabisulfite appears the most economical. Formaldehyde, although effective, is permitted for use in diffusers but not during the refining process.

In diffusers, formalin (30-50% aqueous solution of formaldehyde) is still the most effective agent used to eliminate bacterial populations (Klaushofer *et al.*, 1998). It is added to those cells that are most susceptible to microbial growth as evidenced by low pH. Discontinuous high dosage application of formalin is much more effective than continuous dosing. A beet processing factory will use about 0.25 kg formalin (40% formaldehyde) per tonne of beets processed (Guerin *et al.*, 1972).

Numerous other biocides have been investigated, including sulfur dioxide (Chen and Rauh, 1990), cationic substances (Franchi and Bocchi, 1994), glutaraldehyde (Accorsi, 1994), and hydrogen peroxide (Duffaut and Godshall, 2002). Only the latter seems to provide acceptable results. Mixtures of surfactants with an oxidizing agent such as hydrogen peroxide or peracetic acid have been proposed as well (Bowler *et al.*, 1996).

One aspect of control is related to the choice between microbial production of lactic acid and addition of mineral acid, preferably sulfuric, to diffusion water. Make-up water for continuous diffusers has a pH of 7.0–9.0, and cossettes leaving the tail cell must be at pH 6.0 or less for efficient removal of water by pressing; dried cossettes are used as cattle feed. In some operations, sufficient lactic acid is generated by fermentation to obtain the desired low pH, but lactic acid production during diffusion is not sufficiently controllable or predictable to ensure that the pH of spent cossettes will be <6.0 (Oldfield *et al.*, 1974a).

Control measures to minimize microbial growth and losses in sugar processing are also discussed by Pollach *et al.* (1998), Day (2000), and Trost and Steele (2002).

*Storage stability of final product.* The key requirement is avoidance of moisture uptake, as for cane sugar. In addition, the low ambient temperatures in areas where beets are grown and processed may be responsible for moisture migration or condensation on the product.

### F Microorganisms in refined sugar capable of spoiling other food

Certain microorganisms that grow during extraction and refining can survive the process or gain entrance after processing. Usually there are fewer than 10<sup>2</sup> per g, but if present in sufficient numbers in the refined sugar they may result in serious spoilage in foods that have sugar as an ingredient (Chen and Chung, 1993).

The bacteria most commonly present in sugar are *Bacillus* spp., which do not grow in the raw sugar but may grow in dilute sugar solutions during the refining process. *Desulfotomaculum* (formerly

*Clostridium nigrificans*, *Cl. butyricum*, and thermophilic *Bacillus* spp. may also be present and are of concern if they persist through the refining process and are present in the refined sugar used in canned food.

The bacteria of most concern are the following (Goldoin *et al.*, 1982).

1. *Bacillus stearothermophilus* and *B. coagulans*, which may grow in canned food, producing acid without gas. As the can of container is not distended, the condition is described as “flat sour”, and the two species are designated flat sour organisms. *Bacillus stearothermophilus* is a particular nuisance because it forms spores that are very heat resistant and grows at temperatures up to 75°C. However, growth will not occur at pH values <5.2. In contrast, *B. coagulans* does not grow at temperatures above 65°C and is less heat resistant than *B. stearothermophilus*, but can grow at pH 4.2.
2. *Clostridium thermosaccharolyticum*, which grows well at 72°C, but less well at 75°C. In canned food, it may produce sufficient acid to cause hydrogen swells.
3. *Desulfotomaculum nigrificans*, which grows optimally at 55°C. It may cause sulfide stinker spoilage in canned food.
4. Mesophilic bacteria, yeasts, and molds that can grow at the pH of soft drinks. The most common molds are *Aspergillus* and *Penicillium* spp., whereas species in several other genera are found less frequently (Tilbury, 1968).

#### IV Palm sugar

##### A Initial microflora

The microflora originates mainly from the inflorescences and spathes of the palm or from slits made in the trunk. Microorganisms have been identified as *Acetobacter aceti*, *Aceto. rancens*, *Aceto. suboxydans*, *Leuconostoc dextranicum*, *Lactobacillus* spp., *Micrococcus* spp., *Pediococcus* spp., and *Bacillus* spp. as well as yeasts such as *Saccharomyces cerevisiae* and *Schizosaccharomyces pombe* (Faparusi and Bassir, 1971; Shamala and Skreekantiah, 1988). The pH of the sap is close to neutrality and if concentration is delayed, growth of lactobacilli may rapidly reduce the pH to 4, making conditions favorable for alcoholic yeast fermentation (Faparusi and Bassir, 1971).

##### B Effects of processing on microorganisms

The palms that provide the sap and the processing methods used vary greatly around the world. Generally, the inflorescences are tenderized by bruising, and the sap extracted or sap is collected from slits made in the trunk. Lime is added to the sap to prevent fermentation. After precipitation of the calcium carbonate, the sap is strained and concentrated by boiling, often in open pans. Crystallization occurs and the syrup is poured into molds where it solidifies rapidly (Naim and Husin, 1986; Hamilton and Murphy, 1988). Prolonged boiling destroys vegetative microorganisms, and concentration for crystallization reduces the water activity to 0.80-0.83, preventing the growth of bacteria including spore formers. The product is hygroscopic and molds may grow if it is held inadequately packaged in humid conditions (Naim and Husin, 1986).

##### C Spoilage

Contamination may occur at all points in the process, often leading to spoilage of the sap.

##### D Pathogens

As for section II.

## E CONTROL (palm sugar)

### Summary

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<b>Significant hazards<sup>a</sup></b>	<ul style="list-style-type: none"> <li>• No significant hazard.</li> </ul>
<b>Control measures</b>	
<i>Initial level (<math>H_0</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Reduction (<math>\Sigma R</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Increase (<math>\Sigma I</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<b>Testing</b>	<ul style="list-style-type: none"> <li>• In most situations, testing for <i>Salmonella</i> or hygiene indicators such as coliforms or Enterobacteriaceae is only performed as a verification for the adherence of good hygiene practices during processing and handling.</li> <li>• Testing for specific parameters is performed in special cases, for example, spore-formers in sugar used for canning.</li> </ul>
<b>Spoilage</b>	<ul style="list-style-type: none"> <li>• Growth of xerophilic yeasts possible if <math>a_w &gt; 0.65</math>.</li> </ul>

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<sup>a</sup>In particular circumstances, such as the use of sugar as ingredient for specific products or processes other hazards may need to be considered.

*Comments.* Lime water may be used to control fermentation during extraction, unless the sap is used to prepare a fermented beverage using yeasts. The control of spoilage microorganisms is achieved through the boiling process. Sap collected in the evening may be boiled for preservation and held overnight for further processing with overnight sap. Boiling is also necessary to inactivate invertase, which will invert the sucrose and prevent the setting of the final sugar concentrate in the molds (Naim *et al.*, 1985). Packaging that retards or prevents moisture absorption will prevent the growth of molds on the surface of the crystalline sugar.

## V Syrups

### A Initial microflora

Three major categories of sugar syrups are in use: (i) syrups made by dissolving refined sugar in water; (ii) syrups made by hydrolyzing starches (potato, maize, or wheat) chemically or enzymically; and (iii) naturally occurring tree saps, such as maple syrup.

Sugar syrups or liquid sugars are raw materials manufactured for a wide variety of end users and products, and therefore fulfill different requirements such as concentration, composition and ratio of different sugars, technological properties, and presence of additives (Blanchard and Katz, 1995; Kearsley and Dziedzic, 1995). The initial microflora of liquid sugar is the same as those described in Sections II and III for cane and beet sugar.

Maple syrup is mainly produced in Canada and the North-Eastern United States. Sap drawn directly from maple trees (*Acer. saccharum* or *Acer. rubrum*) is sterile. But under the normal conditions of collection, yeasts and bacteria can be found. Most of the bacteria were identified as *Pseudomonas fluorescens* (Morselli and Feldheim, 1988), which was attributed to poor hygiene during collection. Late season raw sap contained *Pseudomonas*, *Aerobacter*, *Leuconostoc*, and *Bacillus* spp. (Kissinger, 1974). Other studies have shown that the microorganisms in the maple sap were related to those

found on the trees and were *Bacillus* spp. and actinomycetes (Parker *et al.*, 1994). Harvesting conditions and temperatures may be conducive to rapid growth and to spoilage such as green sap due to the development of fluorescent pseudomonads, red sap to yeasts and some bacteria, milky sap to bacilli, and ropy sap to *Enterobacter agglomerans* excreting exopolysaccharides (Britteen and Morin, 1995).

### B Effect of processing on microorganisms

Liquid sugar is refined sugar concentrated after the decolorizing step or made by dissolving crystalline refined sugar in water. It has a sugar content of 66–76° Brix.

Invert syrup is manufactured using microbial invertase, hydrochloric acid, or ion exchange on very acidic cationic resins (Pancoast and Junk, 1980). These processes are performed at temperatures ranging between 65°C (enzymatic) and 90°C (chemical). Starches from maize, wheat, potato, or tapioca are used to manufacture glucose, fructose, or maltose syrups. Details of the processes are provided by Blanchard and Katz (1995), Olsen (1995), and Le Bot and Gouy (1995).

Precise microbiological data on the different types of processes are not available. In view of the processing conditions, no major changes are however likely to occur, except the destruction of vegetative cells and of some spores.

For both liquid sugar and sugar syrups, recontamination with xerophilic yeasts may occur during intermediate storage in tanks, during transport in pipes, pumps, or trucks.

Maple sap is an aqueous solution containing 1–9% sucrose (Morselli and Feldheim, 1988), which is concentrated to obtain syrup and sugar. This is usually done in special pans and molds as described in detail by Salunkhe and Desai (1988).

### C Spoilage

Depending on their sugar content, syrups have a water activity ranging between 0.70 and 0.85, and are therefore prone to spoilage by xerophilic yeasts (Vindelov and Arneborg, 2002).

Different xerophilic yeasts and molds may grow in sugar syrups. *Zygosaccharomyces rouxii*, in particular, is able to grow at  $a_w$  values as low as 0.65 (Tokuoka, 1993). Growth is usually slow because lag times and mean generation times generally are inversely proportional to the  $a_w$ . Bacteria do not grow. Three factors determine growth of yeasts and molds:

- Size of inoculum.
- Availability of nutrients other than sucrose.
- Gradients with increasing  $a_w$ .

Liquid sugars with gradients of  $a_w$  are most susceptible to spoilage because strains of yeast with moderate xerophilic characteristics can grow quickly in solutions with high  $a_w$  (low concentration of sugar); by adaptation or selection they grow across the gradient to the lowest  $a_w$  (highest concentration of sugar). Thus, large populations of xerophilic yeasts may become established in concentrated solutions of sugar. Gradients of  $a_w$  occur because water does not readily mix with highly concentrated solutions of sugar unless agitated. Thus, pockets of water in improperly dried equipment, and condensate that forms on the ceilings and walls of tanks and runs down to the surface of sugar concentrates, provide gradients. Also, improperly washed pipes and valves containing diluted sugar solutions may provide suitable sites for growth of yeasts to large populations.

The presence of xerophilic yeasts is detrimental to the syrup itself and also in products manufactured from these syrups. Development of yeasts may lead to changes in the organoleptic or textural

characteristics of the syrup, while contamination of confectionery products may cause spoilage due to fermentation and gas production (Pitt and Hocking, 1997).

Quality failure of packaged maple syrup through visible mold growth or blown containers from yeast growth may result from improper packaging and storage.

#### D Pathogens

As for cane and beet sugar, this group of products has never been linked to outbreaks of food-borne disease.

The presence of *Clostridium botulinum* was reported in 13 of 1 010 samples of maize syrup (Kautter *et al.*, 1982) at levels of about 50 spores/g. No spores were found in two additional surveys of different types of syrups and products manufactured with this ingredient (Hauschild *et al.*, 1988; Lilly *et al.*, 1991). No cases of botulism have been associated with sugar syrups.

The method of production of maple syrup is not conducive to the survival of pathogens, and at an  $a_w$  of 0.83-0.86 for packaged syrups (Troller and Christian, 1978) the growth of bacterial pathogens is unlikely.

### E CONTROL (syrups)

#### Summary

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<b>Significant hazards<sup>a</sup></b>	<ul style="list-style-type: none"> <li>• No significant hazard.</li> </ul>
<b>Control measures</b>	
<i>Initial level (<math>H_0</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Reduction (<math>\Sigma R</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<i>Increase (<math>\Sigma I</math>)</i>	<ul style="list-style-type: none"> <li>• Does not apply.</li> </ul>
<b>Testing</b>	<ul style="list-style-type: none"> <li>• In most situations, testing for <i>Salmonella</i> or hygiene indicators such as coliforms or Enterobacteriaceae is only performed as a verification for the adherence of good hygiene practices during processing and handling.</li> <li>• Testing for specific parameters is performed in special cases, for example, sporeformers in sugar used for canning.</li> </ul>
<b>Spoilage</b>	<ul style="list-style-type: none"> <li>• Growth of xerophilic yeasts possible if <math>a_w &gt; 0.65</math>.</li> </ul>

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<sup>a</sup>In particular circumstances, such as the use of sugar as ingredient for specific products or processes, other hazards may need to be considered.

*Comments.* The basis for the prevention of spoilage is to prevent recontamination. This is achieved by the application of good manufacturing practice and good hygienic practice including an appropriate hygienic design of the equipment and processing lines. Avoiding the presence of condensation and thus of spots with increased  $a_w$  is of particular importance. Destruction can be achieved using biocides and by steam sterilization of the processing equipment. Recontamination during storage can be reduced by appropriate protection and the use of tanks equipped with air filters and UV lamps (Fiedler, 1994).

In the case of maple, syrup control is essential to achieve the best commercial grade of maple syrups and to prevent deterioration once packaged. Traditionally, maple syrup is packed at the draw-off temperature of 99-103°C directly from the evaporator or finishing pan. Filled containers are laid on their sides to allow syrup to sterilize the head-space and closure. Destruction of spoilage microorganisms can

also be achieved by sterilizing the product before packaging (Dumont *et al.*, 1993) or by UV irradiation of surfaces of syrups during storage (Dumont *et al.*, 1991), ozone, however, has been shown to be of limited use (Labbe *et al.*, 2001).

## VI Honey

The honey bee or hive bee, *Apis mellifera*, makes the bulk of the world's honey. An additional important hive bee in South and East Asia is *Apis cerana* (Crane, 1979). The bees harvest nectar from the nectaries of flowering plants. The sugar content of nectars from different plants varies from about 5% to about 80%, with great differences in the sugars present and their proportions. Evaporation of water in the hive leads to fully ripened honey, with a water content of  $\leq 20\%$ . Chemical alterations occur, particularly the inversion of sucrose to glucose and fructose. The wax cells in the hive are then sealed, preventing the absorption of water and avoiding the risk of fermentation.

Honey is traded in different forms, liquid or crystalline products or a mixture of both, solid crystallized or granulated honey due to glucose crystallization, spreadable creamed honey or comb and chunk honey. The internationally accepted norms for honey are given in the Codex Standard 12-1981 (Codex, 1994).

*Important properties.* The composition of honeys varies widely and depends predominantly upon the composition of the nectar; climatic conditions and extraction procedures have minor influences. The composition also varies greatly between the producing countries, but in general terms the glucose content (ca. 30–35%) is usually lower than the fructose content (ca. 35–45%). Moisture content is usually between 15% and 21%, sucrose about 1–3%, ash between 0.09–0.33%, and pH falls in the range 3.2–4.5 (White, 1978, 1987). The main physical attributes of honey are largely determined by the types and concentrations of sugars. Of major importance with respect to spoilage by fermentation is  $a_w$ .

Table 12.6 shows the relationship between water content and ERH for a typical clover honey (Martin, 1958). Over the temperature range 4–43°C, honey maintained an ERH of  $59\% \pm 4\%$  at a water content of 18%.

*Methods of processing and preservation.* Before extraction, the thin wax coverings or caps that seal the ripe honey in the cells are removed with a heated knife. The cappings still contain a considerable amount of honey, which is recovered by straining, centrifuging, or melting the cappings.

Honey is usually extracted from the uncapped combs by centrifugation. Heather honey, which is thixotropic, cannot be extracted in this way. The honey flows into a tank where coarse wax material is removed by baffles, by skimming, or by settling. It is then strained and piped into containers. Heating

**Table 12.6** Approximate equilibrium between relative humidity of air and the water content of a clover honey<sup>a</sup>

ERH (%)	Water content (%)
50	15.9
55	16.8
60	18.3
65	20.9
70	24.2
75	28.3
80	33.1

<sup>a</sup>From the data of Martin (1958).

is essential at various stages in the extraction and handling of honey, but excessive heat is deleterious, leading particularly to the production of hydroxymethylfurfural which causes darkening and lowers quality (Townsend, 1975). Temperatures of 71–77°C for short periods serve to destroy most xerophilic yeasts present, have little effect on color, and reduce the tendency to crystallization. Details on processing are given by Crane (1979).

#### A Initial microflora

The microbiology of honey has been reviewed thoroughly by Snowdown and Cliver (1996). These authors have underlined the fact that microorganisms of interest to the honey processing industry are those adapted to the characteristics of honey, i.e. high sugar contents, acidity, and the presence of antimicrobials.

The microbial content is, as shown in different surveys, generally low with counts <100 cfu/g, exceptionally up to 1 000 or 10 000 (Snowdown and Cliver, 1996). In most studies, *Bacillus* spp. have been identified as the main microflora, originating from the pollen, nectar, and bee, as well as from external sources such as sugar solutions used to feed bees. *Bacillus* spp. form also the predominant flora in feces from bee larvae and adults, followed by Gram variable pleomorphic bacteria. Molds, actinomyces, Gram-negative rods and yeasts have been isolated as well but not *Clostridium* spp. (Gilliam and Valentine, 1976; Gilliam and Prest, 1987; Gilliam *et al.*, 1988).

Changes occur during ripening of the honey, and vegetative bacteria such as *Gluconobacter* spp. and *Lactobacillus* spp. present at the beginning disappear due to reduced  $a_w$  (Ruiz-Argüeso and Rodriguez-Navarro, 1975; Snowdown and Cliver, 1996).

The microflora of commercial importance are the xerophilic yeasts, which may cause fermentation if the  $a_w$  is sufficiently high, and the spores of bacteria or fungi that are pathogenic to bees or toxigenic to humans.

The most frequently reported yeasts are *Zygosaccharomyces* spp. but a wide range of other genera also occur in unprocessed honey (Tysset and Rousseau, 1981; Snowdown and Cliver, 1996). Molds are found in honey usually at low levels of up to a few hundreds cfu/g, the most frequently isolated being *Aspergillus* and *Penicillium* spp. (Tysset *et al.*, 1970; Gilliam and Prest, 1987). Xerophilic fungi have not been reported. Two spore-forming bacteria, *B. larvae* and *Cl. botulinum*, are uncommon. However, *B. larvae*, the causal agent of “foul brood” or “American plague” of bees, is of high economic importance. Spores of *Cl. botulinum* have been isolated from 7% to 16% of honey samples of various origins at levels ranging between 140 and 80 000 spores/kg (Sugiyama *et al.*, 1978; Huhtanen *et al.*, 1981; Hauschild *et al.*, 1988; Criseo *et al.*, 1993; Lund and Peck, 2000; Nevas *et al.*, 2002). Spores of *Cl. botulinum* appear to survive for long periods in honey (Nakano *et al.*, 1992) and an increased incidence seems to be linked to growth and sporulation in diseased bees in hives (Nakano *et al.*, 1994).

#### B Effect of processing on microorganisms

As extracted from the honeycomb, honey usually has a water content near 18%, corresponding to an  $a_w$  of about 0.60. A strain reported as *Zygosaccharomyces bailii* isolated from honey grew at  $a_w$  0.65 (Leveau and Bouix, 1979), but this was probably more correctly *Z. rouxii*, the only *Zygosaccharomyces* species capable of growth at such low  $a_w$  (Pitt and Hocking, 1997). The minimum  $a_w$  for growth of a group of xerophilic yeasts inoculated into honey was above 0.68 (Esteban-Quilez and Marcos-Barrado, 1976). The heating given to honey after extraction, to control crystallization, provides a pasteurization process in spite of the increased heat resistance provided by the reduced  $a_w$  (Gibson, 1973). Spores of *Cl. botulinum* are not inactivated by such treatments, however.

### C Spoilage

The number of yeasts in honey is usually dependent on the moisture content, increasing as the moisture content increases. Counts up to  $10^6$  cfu/g have been reported (Graham, 1992). *Zygosaccharomyces* spp. are common, particularly the xerophilic species, *Z. rouxii* (Jermini *et al.*, 1987), probably the most common cause of spoilage of honey (Pitt and Hocking, 1997). Another important species of this genus in honey is *Z. bisporus* (Hocking, 1988). Growth of yeasts causes fermentation and leads to unacceptable organoleptic changes.

### D Pathogens

*Clostridium botulinum* in honey has been implicated in several cases of infant botulism (Dodds, 1993; Aureli *et al.*, 2002; Tanzi and Gabay, 2002). Infant botulism has recently been reviewed by Midura (1996) and Cox and Hinkel (2002). As a source of the bacteria causing infant botulism, honey appears to be less important than some other environmental sources (Long *et al.*, 1985). Outbreaks have, in general, been associated with very heavily contaminated honey. For further details, see Kautter *et al.* (1982), Guilfoyle and Yager (1983), Hauschild *et al.* (1988), and Lilly *et al.* (1991).

## E CONTROL (honey)

### Summary

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**Significant hazards<sup>a</sup>** • *Clostridium botulinum*.

#### Control measures

*Initial level ( $H_0$ )* • Presence in honey seems unavoidable. An incidence of 7–16 % and maximal levels of 80 000 cfu/kg have been reported.

*Reduction ( $\Sigma R$ )* • So far no possibilities to achieve consistent reductions in raw honey.

*Increase ( $\Sigma I$ )* • No increase due to the low  $a_w$ .

#### Testing

- Testing for *Clostridium botulinum* is not recommended.
- Testing for sulfite-reducing spores provides information on the general hygiene of honey but low levels or absence will not necessarily indicate absence of *Cl. botulinum*.

**Spoilage** • Growth of xerophilic yeasts possible if  $a_w > 0.65$ .

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<sup>a</sup>In particular circumstances, other hazards may need to be considered.

*Comments.* No practical procedures exist that can prevent the occasional contamination of honey in the hive by spores of *Cl. botulinum* (Hazzard and Murrell, 1989), or that can ensure their destruction in normal processing. Should their elimination be essential, an effective method is heating to autoclaving temperatures, but dilution before processing and reconcentration after processing are probably necessary. Killing effect should in any case be validated.

As recommended by the American Dietetic Association, for prevention of infant botulism, it is important not to use honey as a sweetener in preparations for infants <9–12 months of age (Anonymous, 2003; CDC, 2004).

*Spoilage.* The heating that honey receives during processing should inactivate contaminating xerophilic yeasts, which are the spoilage agents of concern. However, recontamination from equipment and from the air in the processing establishment is common. This can be minimized by adherence to good hygienic practices. Control of any yeasts present after the extraction process depends on the maintenance of an  $a_w$  of 0.65 or below. To achieve this, absorption of moisture must be prevented by appropriate packaging.

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